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Original article:

First report of Cryptococcus gattii in Ecuador

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Abstract

The present is a report of a 37-year-old male patient, who present clinical manifestations of approximately 1 year characterized by high-intensity holocranial headache, photophobia, diplopia, which were exacerbated in the last three months with hallucinations, loss of hearing from the left ear, he reported exposure of 4 years to feces of birds (pigeons) for his work in the seaport.

Who was referred to a private consultation, to assessment and management, were diagnosed with type 2 diabetes mellitus, for which oral metformin was prescribed. Once the treatment had begun, the patient presented exacerbation of the neurological symptoms, which hospitalization of patient underwent research to diagnose his disease, with presumed bacterial meningitis; he underwent lumbar puncture for microbiological studies and began empirical treatment with glycopeptide and carbapenems.

With the antimicrobial scheme, there was a slight improvement in the intensity of its symptoms. In the microbiological study of the preliminary cerebrospinal fluid, , were observed fungal structures compatible with Cryptococcus; a second lumbar puncture was performed to obtain an adequate sample for culture and study with molecular biology, in order to establish the definitive diagnosis of the microorganism.

Keywords: Cryptococcosis; Cryptococcus gattii; Cryptococcus;

Case Report

A 37-year-old male patient, with no history of previous comorbidities, referred to cocaine, marijuana, alcohol and tobacco consumption for approximately 20 years; As for the epidemiological background, he reported close and prolonged contact with birds (specifically pigeon feces) for 4 years, when he was working as a winemaker in the seaport (previous job).

He attended a clinical picture of approximately 1 year of evolution characterized by sudden-onset high intensity holocranean headache, incapacitating to perform daily activities that triggers in abandonment of the work activity that he currently executes (security guard), is subsequently accompanied by photophobia, diplopia and exacerbates the last three months with hallucinations, loss of hearing of the left ear, for which he goes to assessment with neurology, performing tomography and laboratory tests, without evidencing apparent or suggestive lesions of disease, he was transferred to general medicine for metabolic evaluation with based on laboratory results of elevation of lipid profile and diagnostic criteria of type 2 diabetes mellitus, where he began treatment with oral antidiabetics (metformin), however, increases the intensity of headache adding personality disorder, vomiting and horizontal nystagmus in the eye left, accompanied

by diplopia, blurred vision, impaired balance and vomiting on one occasion, after which he went to a new neurological evaluation, and he underwent nuclear magnetic resonance imaging (MRI) of the brain, hydrocephalus was observed, so he was admitted to hospital third-level of the Ministry of Public Health and later referred to a private institution of Guayaquil in October 2018, where on the second day of hospitalization the neurosurgeon places a peritoneal ventricular bypass valve (DVP), without complications, receiving antimicrobial therapy with meropenem and vancomycin studying with good clinical evolution. On the 8th day of the post-surgical period, he presented a thermal increase on several occasions accompanied by deterioration of the sensory, so he required intensive care intubation and hospitalization.

Results

A sample of CSF of cloudy appearance, glycorrhachia 18mg / dl, protein 80mg / dl was studied, no bacteria were observed, but Cryptococcus-compatible capsulated yeasts (Figure 1) were observed at the direct microscopic examination performed with Chinese ink staining technique, Therefore, treatment with fluconazole was initiated. Among the complementary tests, serology was requested for HIV, hepatitis B, and C, proving negative; Normal IgA, IgG and IgM; Serum sputum samples for Bacillus Acid-Alcohol Resistant (BAAR) with negative results, culture of bacteria and mycobacteria without growth, CD4 count of 1380 cells / mm3, chest x-ray without alterations.

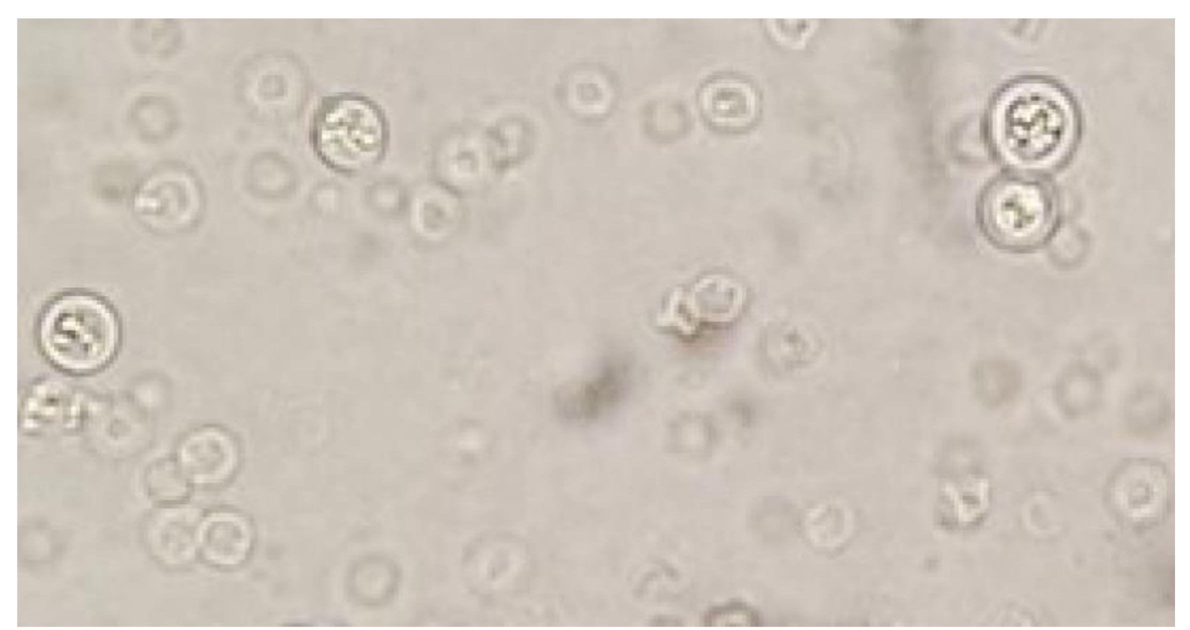


Figure 1. Encapsulated round yeast compatible with Cryptococcus; Source: CSF sample culture, a study conducted at the Microbiological Research Center (CIM).

In the second lumbar puncture in the presence of symptoms, CSF culture was requested, which presented a creamy-bright beige colony on Sabouraud agar, a positive urease test was performed, the yeast was identified as Cryptococcus gattii using the mass spectrometry laboratory technique (MALDI-TOF); culture was carried out in CGB medium, which was positive showing the characteristic cobalt blue color. The confirmation of the diagnosis was made by the amplification of the IGS1e GS2 regions, "Isolated 1112001 showed 100% identity with Cryptococcus gatti, by sequencing the intergenic region (IGS) of the ribosomal 28S RNA gene", establishing the detection of Cryptococcus gattii.

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GAACGGGGTGCTGTAAGTGGTAGAGTAGCCTTGTTGCTACGATCCACTGAGGCT
AAGCCCTTGTTCTATAGATTTGTCTCTAATATGTTAGGTCTCGGGGGGCTTCCTCT
AGAGACTTGGATGTAAGAGGCTTTGCTTTTTTTATGACGATGACTTGTCAAAGT
GTGGATGACTCCAGGAGAGAGGTCGGGCTGGTAATTAGTAATATCAGTCAGTCA
TTTCAGCTGGCGCCATCGATACTTTATAAGTCAAACTTAACCATTTCACTGTGTA
GTAAGTAGTAGGCTCTGAATTACTAGAGACACTTGCCAGGTTATCGCAAGTTGG
CAGGCAGGCAGGACACACATACTATTGATTTCACGGTCATGGGGGACTTGGCTG
CTGGTGCTTGAGTTGCATAATAGAGAAGTATATGCATGTCGCGGGGGGGGGAC
TTGGCTAAGATGCGTTATGCGGCAAGCAAGGTAAGTAGTATTTGTATTAAAGTA
TTTAGTTACTTTTCCAGTCATGGGGGACTTTGATAGTGGTTGTGAGCCGGAATGA
AAAATGGGTAAATGTGGTATGGATGGTGAGAGGGGGAGCTGCCAAGCGTATGC
AGTGGGTGTGCTTATAGCATAGTCAAGAGGGGATGGGCAATTAAATTTTGGAAA
GTTGTTTGACCCGACCTGACGGTGACTCTAATAGGCTGGTCAAGCAAACGTTTA
AGTTGAGTCAAGCTCGGTCAAGCAAAGTCTAGAAAAGATTAATGATCGCTAAGA
ATTACTCCGGTCGCGGGGGGCTTGCAACTTGTCTGGCCCACTG
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>Cryptococcus gattii 28S ribosomal RNA gene and intergenic spacer (IGS)

Table 1. The amino acid sequence of C. gattii identified in the patient's CSF; a study conducted by the Microbiological Research Center (CIM).

The susceptibility profile was determined using the Sensititre Yeast One technique, presenting sensitivity to amphotericin B, fluconazole and voriconazole; therefore, treatment with amphotericin B deoxycholate 1 mg/kg/day intravenous, plus fluconazole.

Discussion

Cryptococcosis is a systemic fungal infection, whose initial focus is the pulmonary parenchyma, which tends to be transient, mild and unrecognized. Cutaneous, bone or visceral forms may occur as a result of the spread of the microorganism by blood count; however, the meningeal form is the most commonly observed of the disseminated forms. The most common etiologic agent

that affects humans is *Cryptococcus neoformans* (vars. *neoformans* and *grubii*), although cases of *Cryptococcus gattii* (especially in immunocompetent) have been reported. These yeast-like fungal agents, which are usually associated with host plants, and whose dispersion occurs with their flowering, in the form of basidiospores, which become yeasts, reaching mammals and birds, who can transmit cryptococci through their intestines and eliminate yeast capped in feces, are wide geographic distribution being considered cosmopolitan. The frequency of occurrence of the disease is sporadic, manifesting as meningoencephalitis and/or pneumonia. [1-4]

Cryptococcus gattii is a fungal pathogen, it is classified according to 4 genotypes (VGI, VGII, VGIII, and VGIV), it is endemic in the tropics and subtropics; in clinical samples it is visualized as simple yeasts with round or cylindrical cells wrapped in a thick polysaccharide capsule, with sporadic reports in the Americas region. The infection is usually acquired by inhalation from the environment, although direct skin inoculation has also been reported, the reasons why cryptococomas are more common with *C. gattii* are unknown, there is increasing evidence that the immune response to Infection between immunocompetent hosts plays an important role. [2-7]

Historically, phenotypic and biochemical tests are frequently used in developing countries for the identification of species, however, these analyzes require a lot of time and are prone to errors. The limited use of MALDI-TOF MS and Sanger sequencing in developing countries is due to high costs. Arastehfar et al. developed a panel of PCR assays, the YEAST PANEL multiplex PCR assay, which identifies the most clinically important yeast species, which include *Cryptococcus spp.* [8]

The incubation period for C. gattii infection in humans is uncertain, in a 2006 study, in seven travelers to Vancouver Island, an average time of six to seven months (range of 2 to 11 months) was determined as with C. neoformans, HIV-infected patients with C. gattii infection present mostly meningoencephalitis. Symptoms such as fever, chills, and weight loss have been reported within the systemic characteristics, with fever being the most frequent. In addition to the condition of the central nervous system, eyes and lungs, C. gattii can also affect the skin, soft tissues, bones, joints, bone marrow, larynx, and lymph nodes (intra-abdominal form is rare). The cutaneous form can occur together with the pulmonary and CNS infection or as a primary skin disease; In contrast to C. neoformans, patients with C. gattii are more likely to have cryptococcomas in the brain and lungs and are often HIV negative. [3, 5]

The treatment of *C. gattii* infection is based mainly on expert opinion, there is no data from randomized trials regarding the treatment of *C. gattii* infection, and the optimal management strategies are not yet defined. The differentiation of species is currently relevant to better understand its relationship with geographical particularities, preference clinics and host results. [4, 6]

In the study by Damasceno-Escoura et al (2019), 11 *C. gattii* were identified in patients from a non-endemic area of Brazil for this microorganism, 4 infected with HIV, 1 kidney transplant, 1 patient with a decrease in cause CD4 + T lymphocytes unknown, 1 with chronic liver disease and 4 immunocompetent. Marr et al. (2019) described 88.2% of cerebrospinal fluid cultures positive for cryptococcus antigens, in a sample of 54 patients, while only 5 patients out of 28 had rescue of C. gattii in bronchioalveolar lavage samples. [6, 7, 9]

For all patients with *C. gattii* infection of the central nervous system (CNS), induction therapy with a formulation of amphotericin B plus flucytosine are recommended. Amphotericin B deoxycholate (0.7 to 1.0 mg/kg intravenously [IV] once a day) is often used, but if there is nephrotoxicity, lipid-based amphotericin B formulations such as liposomal amphotericin B (3 to 4 mg/kg daily intravenously) or the amphotericin B lipid complex (5 mg/kg daily intravenously) seems to be equally effective [4].

The consolidation regime of choice to prevent relapse is fluconazole, instead of another azole, it should be administered at a dose of 400 mg orally daily for eight weeks, followed by a maintenance dose of 200 mg orally daily for approximately 12 months to eradicate the infection. The total duration of antifungal therapy will depend on the clinical response and mycological negativization; Many professionals extend the duration of maintenance therapy beyond 12 months for cure (mean of 18-month antifungal therapy, range of 8 to 60 months) [4].

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